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REVIEW ARTICLE

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Human Amniotic Membrane: A review on tissue engineering, application, and storage

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Abstract

Human amniotic membrane (hAM) has been employed as scaffolding material in a wide range of tissue engineering applications, especially as a skin dressing and as a graft for corneal treatment, due to the structure of the extracellular matrix and excellent biological properties that enhance both wound healing and tissue regeneration. This review highlights recent work and current knowledge on the application of native hAM, and/or production of hAM-based tissue-engineered products to create scaffolds mimicking the structure of the native membrane to enhance the hAM performance. Moreover, an overview is presented on the available (cryo) preservation techniques for storage of native hAM and tissue-engineered products that are necessary to maintain biological functions such as angiogenesis, anti-inflammation, antifibrotic and antibacterial activity.

KEYWORDS

cryopreservation, cryoprotective agent (CPA), extracellular matrix (ECM), human amniotic membrane (hAM), scaffold, tissue engineering (TE)

1 | INTRODUCTION

Tissue Engineering (TE) is an interdisciplinary field involving process engineering, material science, biology, chemistry, physics, and medicine, which focuses on the development of tissue analogs aimed at supporting cellular responses towards successful regeneration of lost or damaged tissues and organs. TE includes cells, scaffolds, and growth factors or biomolecules to induce tissue growth. The basic idea behind TE is the production of a new functional scaffoldless or scaffold-based tissue structure, which can be in the form of tissue-engineered constructs (TECs) or tissue engineered products (TEPs) as commercialized TECs alternatives.

TECs are obtained by the colonization of a scaffold with cells, which should be able to proliferate, differentiate, and replicate the cell/tissue function. The scaffold acts as a platform for cell seeding and should allow cell infiltration for the development of a functional tissue.[1,2] Hence, the cells require the development of a biocompatible scaffold from native human tissue, synthetic material, or a combination of the two that mimics the extracellular matrix (ECM).[1,3,4] The ideal scaffold should be biodegradable, meaning that once transplanted or inserted into the native tissue environment, it should degrade in a non-toxic manner being replaced by native components once tissue regeneration is completed and normal physiological functions are restored. Another important TE process is the storage of the TEPs, which is an inevitable step in ensuring their on-

demand availability for clinical applications and testing of novel therapeutics.

In order to obtain an appropriate scaffold, many types of native tissues can be used as scaffolding material. One of the most explored tissues is human fetal membranes, (e.g., human amniotic membrane [hAM] and chorionic membrane), whose clinical use dates back to the beginning of the 20th century when the amniotic membrane was first used as superficial skin dressings.[5] Moreover, being a readily available biocompatible material, these membranes could be collected at the time of birth after informed consent. hAM consists of outstanding structural and regenerative-associated components, such as growth factors, collagen, and glycoproteins, which can be used for wound healing, and tissue re-modulation or regeneration. However, some challenges arise for specific applications, mostly due to the mechanical stability, differences between donors, and changes in the membrane properties related to the preservation method.[6] In this review, recent studies on the application of native hAM as a scaffold and hAM-based TECs are discussed. Moreover, an overview of preservation techniques for hAM is presented. In a general search in Scopus with the keyword "hAM+scaffold" a total number of 279 publications (period 2000–2020) were found with an increased trend in the number of publications over the last 10 years. This trend could be related to an increase in TE research in the last two decades. However, within these 279 publications, there is a noticeable mismatch among the number of publications related to hAM TE and the number of publications elaborating on their storage methods (keywords "hAM+storage+scaffold"), for example, only seven storage-related publications within the last 10 years were found. Finally, with the keywords "hAM+storage," a total number of 95 publications classified from 2000 to 2020 were found with an increasing rate of publication in the field over the last 5 years as shown in Figure 1.

1.1 | Human amniotic membrane

The amniotic and chorionic membranes (i.e., also referred to as amnion and chorion, respectively) are part of the fetal portion of the placental membranes. The amnion is in contact with the fetus, while the chorion is adjacent to the endometrium (maternal tissue).[7] hAM is the innermost layer of the placenta with a thickness between 20 and 50 μm and consisting of five different layers, as described in Figure 2(a): a single layer of epithelium, a basement membrane, a compact layer, a fibroblast layer, and a spongy layer. The basement membrane contains collagen Types IV and VII, fibronectin, laminin, as well as hyaluronic acid secreted by the epithelial layer.[8-10] The compact layer is composed of collagen Types I and III synthesized by mesenchymal stem cells, whereas the fibroblast layer contains macrophages. Lastly, the spongy layer allows the hAM to slide upon the chorion.[11,12] The hAM and the chorion form the fetal membranes, and both layers can be separated as shown in Figure 2(b).

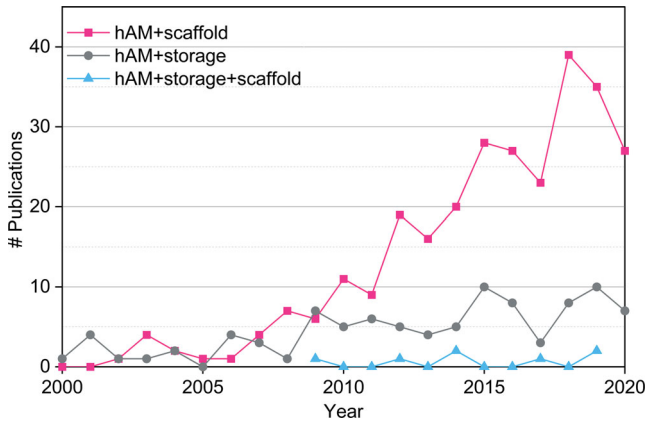

FIGURE 1 Number of publications in the period 2000–2020 related to the keywords hAM, scaffolds and storage

Since 1910, hAM has been used for therapeutic purposes such as skin transplantation.[13,14] The first application of hAM in ophthalmology was described in 1940 by de Röth to repair symblepharon and conjunctival defects.[15] In addition, hAM became in the second half of the 20th century one of the first biomaterials to be used in the development of tissue-engineered constructs supporting cell migration and growth of new tissue.[5,10] Recently, since 1995 reports on its application in TE have increased with hAM being used as a biomaterial for soft TE in the fields of dermatology, plastic surgery, skin transplantation, and as a biological dressing for ophthalmic healing.[8,10,16] Moreover, hAM continues to gain traction as a novel material in the cardiac field due to its outstanding properties as a scaffold for blood vessels[9] and as a pericardial substitute.[17]

1.2 | Biological properties

hAM has proven to be an outstanding scaffold for TE owing to its ability to allow the transport of water and the presence of growth factors such as the epithelial growth factor.[8] hAM has advantageous characteristics including: (a) anti-inflammatory effects owing to the production of anti-inflammatory factors such as hyaluronic acid; (b) suppression of pro-inflammatory cytokines; (c) anti-bacterial properties due to molecules such as β-defenses and elafin[12]; (d) anti-fibrotic properties due to the down-regulation of TGF-β and its receptor expression[10]; (e) low antigenicity[18]; as well as (f) immunomodulatory properties as a result of the factor secreted by the epithelial cells, which inhibits the migration of macrophages and natural killer cells to prevent a maternal immune attack.[12] So far, two types of cells can be isolated from the hAM: amniotic epithelial cells (AEC) and amniotic mesenchymal stem cells (aMSC) derived from the epithelium and stromal layer. Further information on the biological characteristics of amnion derived MSCs and applications can be found in detail in the review by Parolini et al..[19]

1.3 | Mechanical properties

The mechanical properties of hAM, such as elasticity, stiffness, and tensile strength are related to the composition of the placenta. The orientation of the collagen fibrils in the ECM is responsible for the tensile strength, whereas the elastic deformation is related to the presence of elastin fibers,[12] laminin, hyaluronic acid and glycosaminoglycan.[7] Some research has shown the amniotic membrane shear modulus to be between 100 and 400 Pa, with the difference in measurements related to the state

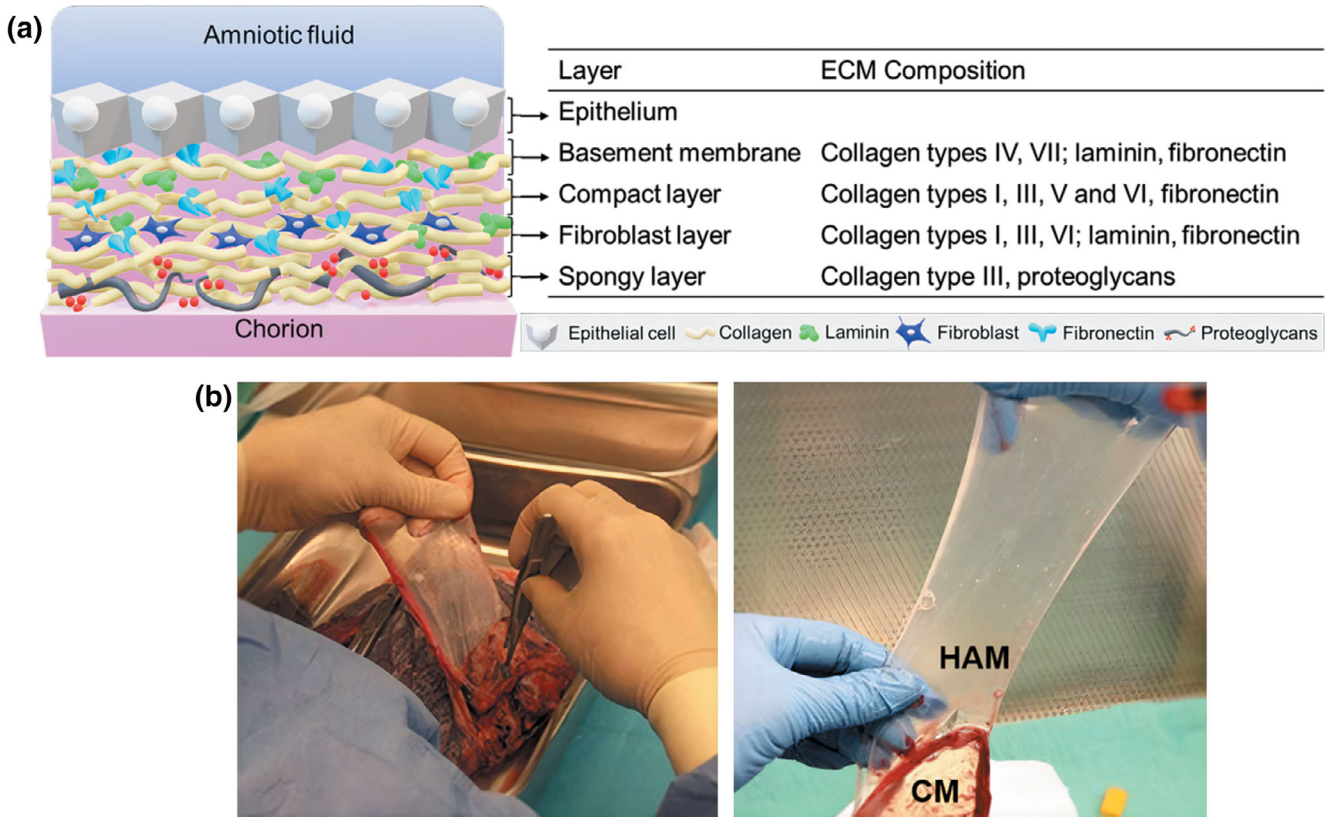

FIGURE 2 Human placental membranes, (a) Schematic representation of the structure of hAM, and the composition of extracellular matrix for each layer. (b) General appearance of the human fetal membranes (left image). The hAM is a thin translucent layer attached to the chorionic membrane (CM) (right image)

of the hAM used.[20] Decellularized hAM presents higher shear modulus than native one, because the denudation process dehydrates the membrane and thus decreases its thickness. It has also been shown that the elastic modulus decreases with increasing hAM thickness.[20,21] Moreover, the reported values of elastic modulus differ between hAM from distal (~135 Pa) or proximal parts (~62 Pa) in the placenta,[20] which could be attributed to the thickness of the membrane, the tissue composition, and to a time-dependent viscoelastic property of creep of the hAM. This viscoelastic property is related to the increase in the amniotic fluid volume and fetal development during gestation.[12]

2 | HAM PREPARATION

2.1 | Collection and preparation

In general, hAM is obtained from the human placenta, which is collected by medical staff from pre-selected donors after the cesarean delivery with informed consent. hAM could also be collected from vaginal delivery; however, in this case, there is a risk of contamination by normal flora during the process. For this reason, the collection under strict aseptic conditions after cesarean delivery is preferred. Following the collection, the placenta is stored in a sterile container and transported for processing. The transport time should be as short as possible with a recommended maximum time of 24 hr. If the transportation time is less than 2 hr after the delivery, the placenta may be transported at room temperature. Otherwise, for transportation longer than 2 hr, the temperature may not exceed 2–8°C. In this case, a sterile solution such as saline solution or phosphate-buffered saline (PBS) should be placed in the container to protect the tissue from dehydration.[22]

Prior to preparation, the placenta is decontaminated to remove pathogens. This may be performed by rinsing with sterile saline or PBS, and in some protocols with antibiotic/antimycotic solution. The blood clots present in the membrane are afterwards removed through rinsing, and finally, the amnion and chorion are separated manually (Figure 2(b)). The hAM is washed with saline solution, cut in pieces of the desired size, and placed on a carrier such as nitrocellulose.[8] Proper screening is required to test for infectious diseases such as human immunodeficiency virus (HIV) Type 1 and 2 antibodies, Hepatitis C antibody, Hepatitis B surface antigen, Hepatitis B core total antibody, serological test for Syphilis, HIV Type 1 nucleic acid test, and Hepatitis C virus.[23] The requirements for testing may differ depending on local or regional guidelines and directives.

2.2 | Decellularization

As described previously, hAM contains fibroblasts, mesenchymal stem cells, and a layer of epithelial cells. The non-decellularized hAM shows appropriate performance as a scaffolding material, with only a few studies showing a slight immunogenic response that caused inflammation in the

treated tissue.[24,25] Decellularization helps eliminate possible immunogenic responses by removing the cells and cellular debris from the amniotic membrane while leaving the extracellular structural proteins intact. Several decellularization methods using ethylenediaminetetraacetic acid (EDTA), trypsin, dispase, urea, Triton-X100, and thermolysin exist and have been applied to date. The success of the decellularization process is analyzed with optical microscopy, fluorescence staining or DNA quantitative essay. Moreover, the adequate preservation of the ECM in the decellularized hAM improves the interaction of different cell types with the membrane.[17,18,26,27]

3 | HAM SCAFFOLD TISSUE ENGINEERING

3.1 | Challenges of native hAM

Despite its excellent biological characteristics, the use of native hAM poses significant challenges due to differences in the content of growth factors and regulators for the ECM remodeling, the region of the membrane, and the delivery method among donors.[28]

Furthermore, the low biomechanical consistency, rapid biodegradation,[27] and the large amount of expensive testing that is required from donors to ensure that the transmission of diseases does not occur hinders the use of native hAM.[10] Besides, even after testing, there might be complications during its usage due to the transmission of bacterial, viral, or fungal infections. In relation to the former, the rates of postoperative contamination reported in the literature are contradictory, with the experience of most authors[29] (5000 transplants), and the *German Society for Tissue Transplantation* (DGFG) (6000 transplants) showing no associated contamination, while other authors[8] indicate values to be between 1.6 and 8%. In addition, Notify library has reported only three microbial contamination adverse reports of medical products with hAM since 1998; however, none of them are used for transplants.[30]

Other secondary complications that may occur during the application of hAM are related to inflammation. Despite the reports about hAM's anti-inflammatory properties, some research groups have indicated a minor inflammatory response in histological sections of wounds grafted with hAM.[31] In addition, the storage method of hAM should be followed with extra care as some studies have shown a reduction in hAM cell viability (if high cell viability is required) after thawing, especially for periods longer than 6 months.[32]

For the reasons outlined above, TE of amniotic membranes provides an excellent alternative to establish scaffolding material with more homogenous properties than native tissue. In the following subsections, the primary methods to produce scaffold materials for TE are further described and discussed.

3.2 | Scaffold fabrication techniques

Several approaches are employed to fabricate scaffolds for TE. The ideal technique, however, depends on the desired properties of the tissue to be regenerated. For instance, allogeneic grafts are used as a replacement for the patient tissue. Other techniques involve synthetic materials to create scaffolds with desired properties. The most used methods with their advantages and disadvantages, as well as the incorporation of hAM, are described below.

3.2.1 | Allogeneic grafts

Allogeneic grafts are off-the-shelf tissues in which the natural ECM acts as a mimic for the native tissue to be replaced. Depending on the source, their availability can be less limited, such as it is in the case of fetal membranes which are usually treated as waste.[33]

In contrast, the implantation/transplantation of allogeneic grafts between donors and recipients may trigger an immunological response. To decrease their immunogenicity, allogeneic grafts can be decellularized to remove cells and cellular components while retaining the properties of the ECM,[1] as it has been conducted with proven success, for example, for heart valves.[34] Due to the unique properties of hAM, decellularization may not be necessary, as hAM is considered to be an immune-privileged material which rarely causes deleterious allogeneic immune reactions.

Likewise, hAM has been used as an allogeneic graft for the treatment of skin, eyes, cartilages, nerves, and blood vessels. For oral and periodontal tissues, for instance, one advantage of hAM graft is the adaptability to different cavity morphologies. However, the degradation of hAM in the oral environment together with the mechanical properties required to resist masticatory forces are considered as a challenge.[23]

In TE of blood vessels (TEBV), hAM reduces the development time of the scaffold as it can be used directly as a matrix for endothelial cell culture in a one-step fabrication process. Moreover, in TEBV, the scaffold exhibited good mechanical response to simulate physiological shear stress (12 dyne/cm^2, similar to arterial stress) for 4 days. Although the properties of native hAM are adequate for this kind of application, the remodeling process in the hAM-based TEBV should be studied in more detail.[9] Furthermore, hAM used in clinical trials as a graft in the treatment of human burns showed enhanced healing in comparison with atopic treatments (average healing with hAM around 13 days and an average of 23 days healing atopic treatment).[35]

3.2.2 | Hydrogels

Hydrogels are frequently employed as scaffolds because of their three-dimensional crosslinked hydrophilic polymeric network structure that can absorb and retain water. This hydrophilic characteristic is also present in fetal membranes in which hAM acts as a membrane to transport water-soluble components.[1] Murphy et al. 36 proposed a hyaluronic acid hydrogel with the inclusion of solubilized amniotic membrane (containing cell-derived cytokines and growth factors) for effective treatment of wounds, assuring wound closure, and healing with cosmetic outcomes. They demonstrated the efficacy of the novel material as a wound treatment in a murine model as the material accelerated wound closure and prevented wound contraction after 14 days of implantation.

Nonetheless, the rapid degradation of hydrogels due to its components is difficult to control; it becomes much faster in aqueous environments such as body fluids. Cell penetration and lack of vascularization are additional challenges for this type of approach.[37]

3.2.3 | 3D printing

The technique of 3D printing, or rapid prototyping, is one alternative to control the scaffold morphology and appearance through layer-by-layer deposition of a curable polymer. 3D printing has been used as an alternative for building scaffolds intended to replacing hAM in ocular defects. Dehghani et al.[31] proposed a 3D printed membrane using a gelatin, elastin, and sodium hyaluronate blend for conjunctival reconstruction as an alternative to hAM. The 3D membrane exhibited optimal physical and mechanical characteristics for ocular surface/conjunctival construction and as shown in a rabbit model, presented a more predictable degradation pattern, less inflammation, and reduced scar tissue formation in comparison with native hAM. To current knowledge, direct 3D printing of hAM has not been established yet. Skardal et al.[38] printed amniotic fluid-derived stem cells resuspended in fibrin-collagen gel over the wound site of injuries in mice skin showing better-wound closure and re-epithelization after 14 days as compared to pure fibrin collagen gel. One of the main disadvantages of 3D printing is the impossibility to reach sizes in the nanometric range (100–500 nm) in the structural elements comparable to those elements in the ECM.

3.2.4 | Electrospinning

Electrospinning is an outstanding technique to mimic the native ECM allowing for the fabrication of scaffolds with nano-to-micron diameter fibers, similar to the size of fibers found in the ECM such as collagen (500 nm) and elastin (400 nm)[39] also providing a functional environment for cell growth and tissue regeneration.[3,40] Previous studies of decellularized hAM presented a tissue structure similar to the one that can be obtained through manufacturing techniques such as electrospinning.[17] In the electrospinning process, a polymeric solution is contained in a syringe. The solution gets charged by connecting a power source to a blunted needle placed at the outlet of the syringe. The charged solution is then pumped at a defined rate towards a grounded target (collector). The final electrospun membranes have a similar pattern to the structure present in fetal membranes, as shown in Figure 3.

Szentivanyi et al.[41] and Suresh et al.[42] discussed the effect of several parameters of the electrospinning process on the scaffold's fibers, pore size, and porosity, which are critical to produce scaffolds with characteristics close to ECM. The parameters and properties influencing the scaffold properties can be divided into process parameters (voltage, flow rate, distance), environment parameters (processing temperature, humidity, partial pressure) and solution properties (viscosity, conductivity, surface tension).[43] Among the process parameters, applied voltage (in kV), spinning distance (in cm) and flow rate (in ml/hr) have a significant influence on fiber and pore size as well as on the scaffold porosity. By changing the electrospinning time, scaffold materials with varying thicknesses can be generated and thus can be adjusted for the aimed application. Coaxial electrospinning, which is one modification of an electrospinning process, was also described as an option for the fabrication of scaffolds with fibers possessing a core-shell structure as presented in Figure 4. For this process configuration, different biocompatible polymers could be employed for the core and shell to obtain a composite fiber scaffold that mimics biological tissue and that allows the controlled release of active substances, such as growth factors.[44]

Polymeric scaffolds produced using the electrospinning technique are also considered as promising materials for cardiovascular,[45] bone,[46] and nerve[47] TE. The conventional materials for electrospinning scaffolds are polymers and blended polymers, including collagen. In addition to the application of native hAM for corneal treatment[8] information about polymers for corneal TE can be found in detail in the review by Kong et al..[48]

One schematic proposal to obtain a tissue-engineered amniotic membrane by electrospinning through the mix of polymers with hAM

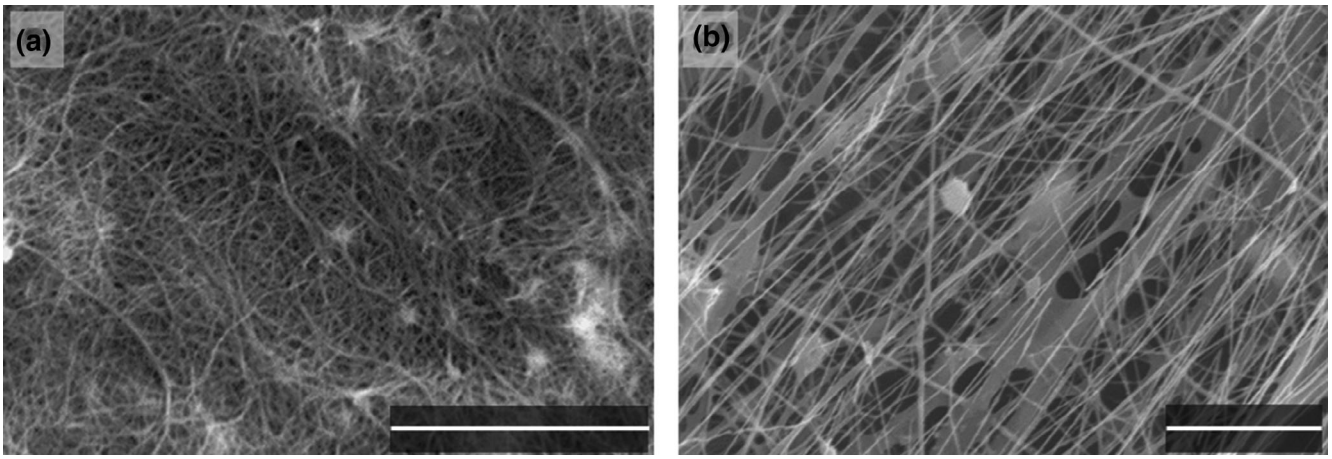

FIGURE 3 Scanning electron microscopy images of native human fetal membrane (a) and porous fibrous network obtained by blend electrospinning configuration with 5% w/v polyethylene oxide and hAM powder (b). Scale bars are 10 μm. The analyzed size of the fiber is 243 nm ± 67 nm for a and 275 nm ± 84 nm for b

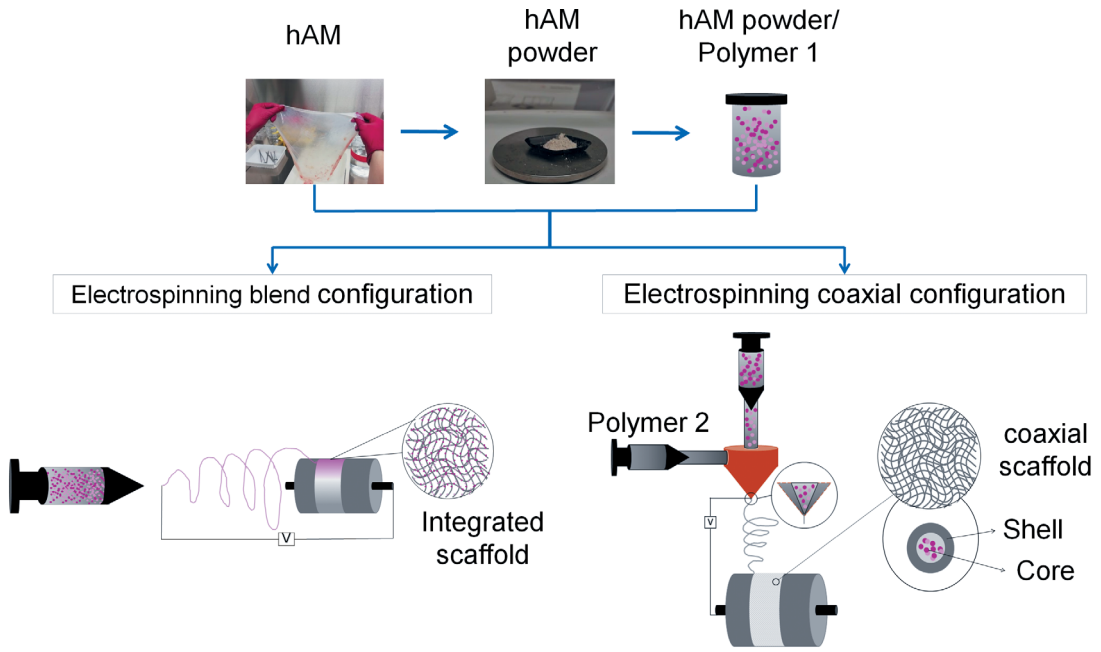

FIGURE 4 Schematic representation of a process proposal to obtain tissue-engineered membranes with hAM by blend and coaxial electrospinning configuration

TABLE 1 Application of hAM composites and commercial products

Application	Form of hAM	Membrane material	In vitro test	In vivo test	Reference
Wound healing and tissue regeneration	hAM lyophilized and pulverized	Scaffold with a synthetic polymer and a natural polymer	Keratinocytes and fibroblast	Skin model nude mice and Yorkshire pigs	49
	hAM solubilized	Hyaluronic acid hydrogel		Mice	36
Reconstructive urology	hAM frozen	Electrospun poly-(L-lactide-co-E-caprolactone)	Mesenchymal stem cells	Wistar rats	50
Artificial cornea material - Keratoprosthetic	hAM decellularized	Polyvinyl alcohol (PVA)	-	Rabbit corneal epithelial cells	51
Aligned tissue regeneration	hAM decellularized	Electrospun fibers of PLGA	-	Skeletal muscle cells	52
Ocular surface reconstruction	*Amnioguard* (bio-tissue)	N/A only native hAM	Clinical test 11 patients		53
Neuropatical corneal pain	*ProKera* ®(bio-tissue)	N/A only native hAM	Clinical test 9 patients		54
Ocular surface disorders	*AmnioClip-plus (DGFG)*	N/A only native hAM	Clinical test 7 patients		55
Premature rupture fetal membrane	hAM cell-free scaffold	Polyester urethane scaffold used as a comparison	-	Rabbit model	56
Vascular graft	Rolled hAM	N/A only native hAM	-	Rabbit model	57
Tendon regeneration	hAM pulverized	Collagen-glycosaminoglycan Hyaluronic acid	Tenocytes	-	58
Skin regeneration	hAM decellularized	Nanofibrous silk fibroin	Adipose tissue-derived mesenchymal stem cells	-	59

powder is presented in Figure 4. This scheme shows both blend and coaxial configurations. The hAM powder is obtained in two steps: (a) after collection, the native hAM is freeze-dried to ensure the preservation of the ECM during the storage, and (b) the dried hAM is then milled using a cryomill to obtain small particles that can be mixed with the polymer solution for fabrication of scaffolds.

3.3 | Composites

The hAM is an outstanding allogeneic graft material. However, to improve the variability of its mechanical properties (as discussed in Section 1.3), and the variation of biological performance (related to storage conditions or donor variability) some works and patents have discussed employing complementary scaffolding techniques with hAM inclusion as summarized in Table 1, with the aim being to create composites utilizing biomaterials that enhance the hAM scaffold efficacy for different applications.[11]

In one study, lyophilized and pulverized hAM was combined with synthetic and natural polymers to develop composite scaffolds for TE. Such scaffolds showed good responses for skin regeneration with a better cosmetic appearance in comparison with native hAM alone in an animal model.[49] In another study,[50] frozen hAM was covered on both sides with two-layered membranes produced by electrospinning from poly-(L-lactide-co-ϵ-caprolactone) to form a sandwich structure. The sandwich biocomposite was tested in bladder augmentation in Wistar rats with hemicystectomy as a model for reconstructive urology. The regeneration of the urothelial and smooth muscle layers was adequate, and the biocomposite improved its mechanical resistance.

Uchino et al.[51] performed the decellularization of pieces of hAM (2 x 2 cm^2) and lyophilization before immobilizing it with polymers. This kind of composite was employed in a rabbit model as an artificial cornea material. In this research, hAM immobilized with polyvinyl alcohol hydrogel (PVA-AM) was compared with PVA and collagen (PVA-COL) when seeded with rabbit corneal epithelial cells. Results showed an improved corneal epithelialization on PVA-AM in rabbit corneas compared to PVA-COL, suggesting the possibility of using PVA-AM as a biocompatible hybrid material for keratoprosthetics.

In another study, scaffolds with PLGA fibers on hAM were evaluated for aligned tissue regeneration of skeletal muscle cells, and the results showed appropriate orientation of the cells along the PLGA fibers with viability and migration rates similar to cell seeding just on hAM. The composite scaffolds presented good mechanical strength and elasticity with potential use in the development of aligned tissue constructs.[52] In general, composites with hAM presented better results than native hAM alone.

3.4 | Commercial products

The U.S. Food and Drug Administration (FDA) regulates different products containing human fetal membranes in the United States of America under the tissue guidelines (HCT/P 361). In accordance with these guidelines, the products must satisfy the following criteria: minimal manipulation, not being combined with drugs or devices, and not being reliant on cell metabolic activity as a primary function. Placental-derived allograft tissue is available in two principal forms: sheets consisting of one or more intact layers of the placental membrane; and injectable products, such as amnion suspension allografts (typically composed of micronized amnion).[7,60,61]

The company *AmnioChor* (USA), a stem cell bank, in 2017 listed 116 companies with 139 products on the hAM tissue market of which 68 contain amnion, 11 amnion+chorion, 23 amnion+composite and 37 amnion flowable solution.[62] This listing includes companies as *Bio-Tissue* (USA) with three products of cryopreserved hAM, and *IOP ophthalmic* (USA) with at least three products of freeze-dried hAM for ophthalmic applications.[62,63] Also, *The Musculoskeletal Transplant Foundation* (USA) produced allografts of hAM and chorion,[64,65] and *International Bioimplant Co* (Iran) produced decellularized hAM patches,[66] involved in clinical trials for the treatment of diabetic foot ulcers.[65] In the same year, the number of worldwide research clinical trials involving hAM were 114 and the number of research projects were more than 1900.[67]

In the European Union, the European Commission for public health established in 2004 the Commission Directive 2004/23/EC for the donation, procurement, testing and distribution of human tissues and cells including hAM and other non-reproductive tissues. Under this guideline, and under the directives 2006/17/EC, 2012/39/EU, and 2006/86/EC, more than 10,000 hAM (from 330,128 non-reproductive tissues) were distributed in 2017 (between January 01, 2017 and December 31, 2017) among 4500 recipients in 25 countries of the EU.[68] Between 2011 and 2018, the European Medicines Agency (EMA) delivered scientific recommendations to classify 13 tissue-engineered products, which could contain hAM or derivate as aMSCs, as advanced therapies for the treatment of burns, scars, or non-healing wounds.[69]

The European Commission reports in the EU Tissue Establishment compendium a total number of 172 institutions (between biobanks and private institutions) that could process, preserve, store, or distribute amniotic membrane.[70] However, the given information is not enough to sort out the institutions depending on whether they produce or only distribute the hAM for patients.

Concerning commercial products, *Keera SRL* (Italy) currently produces a lyophilized extract of fresh human hAM for ophthalmic applications,[63] and the *Barcelona Tissue Bank* (BTB) produces freeze-dried hAM extract for uses in ophthalmology in patients with epithelial defects of the cornea.[71] Several tissue banks produce fragments of hAM on different carriers available in different sizes like the *BTB*,[72] or the non-profit organizations *German Institute for cell and tissue replacement* (DIZG) and the DGFG.

4 | STORAGE

4.1 | Overview of preservation techniques

One of the main challenges in ensuring the availability of fresh hAM, commercial products, or TEPs is the selection of an appropriate storage method. Preservation of clinically relevant cells and tissues is vital to guarantee their continuous supply for clinical applications. In this regard, various preservation techniques have been used to preserve viable and functional cells, tissues, and biological constructs, including the application of protective solutions, cooling to the required storage temperature, and their storage at the given temperature. High cell

viability and functionally as well as tissue stability are the key factors for preservation outcome, and therefore, materials should be preserved using the most optimal preservation procedure. Among the storage processes, fresh storage at subzero temperatures (storage at 4°C), slow freezing, and vitrification are the preferred methods when cell preservation is required. On the other hand, freeze-drying, and air-drying, among others, are commonly used for preservation of the extracellular matrix. Further information on cryopreservation can be found in detail in the reviews by Jang et al. and Hunt.[73,74]

With an application of cryoprotective agents (CPAs), freeze–thaw procedures, adequate storage temperatures, and cryopreservation are used to stabilize biological materials over long periods.[75] The type and concentration of the CPA and the freezing/thawing rates are the most critical process parameters affecting the integrity and functionality of hAM. CPAs reduce freezing damage as they affect water transport, nucleation, ice crystal growth, and stabilize the cell membrane and tissue structure.[74,76] CPAs can be divided in two types: intracellular and extracellular, depending on their capacity to permeate the cell membrane.

Conventional permeating cell membrane CPAs include dimethyl sulfoxide (DMSO) and alcohols, such as ethylene glycol and glycerol. Permeating CPAs, such as DMSO, have been used for decades in cryopreservation of cell transplants.[77] However, there are some concerns regarding the effects of DMSO on cell biology and apparent toxicity in patients.[77,78] Among the extracellular CPAs, the most commons are 2-methyl-2,4- pentanediol, polymers such as polyvinylpyrrolidone, sugars, and sugar alcohols such as sucrose, trehalose, mannitol and sorbitol. Both types of CPAs have been used since the first indication of the cryoprotective properties of glycerol,[79] DMSO,[80] and sucrose.[74,81,82] Techniques such as electroporation-assisted delivery of sugars have also been explored for the development of storage strategies. The latter does not include the application of DMSO for prospective storage of cells and TECs, resulting in equal or even increased viability rates.[83] Due to the complexity of tissues, each method affects its long-term properties differently. The most used preservation approaches are addressed in the following subsections.

4.1.1 | Slow freezing

During slow or equilibrium freezing, the water within the cytoplasm is initially replaced with permeating CPAs to reduce cellular damage. The slow cooling associated with this technique facilitates the intracellular water efflux from cells which allows to eliminate supercooling. Such elimination reduces the damaging effects of intracellular ice formation during freezing, and its recrystallization upon thawing.[84] Protocols for slow cooling commonly use cooling rates in the range of 0.2–10 K/min with less than 10% w/v CPAs. High stability of the cooling rates is of great important during slow freezing; therefore, the use of controlled-rate freezers is necessary for this approach. Slow freezing is commonly conducted down to −80°C following transfer either to −150°C freezers or into a liquid nitrogen phase. Although this technique is easy to handle, it requires the use of high-cost controlled rate freezing equipment for optimal results.[74] Novel approaches for slow freezing are detailed in Section 4.2.

4.1.2 | Vitrification

Vitrification is a process by which the aqueous phase is transformed directly to a glass phase after contact with liquid nitrogen. This technique is advantageous over slow freezing because of the formation of a vitreous (glassy) state during the vitrification process.[85] Nonetheless, in this process, the use of high concentrations of cryoprotectants (40–60% w/v) is required to achieve a vitreous state. The application of high concentrations of permeating CPAs in this technique, such as DMSO, ethylene glycols, glycerol may result in high toxicity which could lead to genetic and epigenetic alterations in cells, such as DNA methylation, and post-translational histone modifications, as reviewed by Chatterjee et al..[86,87] Besides, excellent manipulation skills are required for this process, and there is a high probability of contamination.[74] Vitrification is employed mainly to preserve germplasm (reproductive cells such as oocytes and sperm), although it has also been applied for the preservation of epithelial cells in the hAM and the preservation of mesenchymal stem cells derived from hAM. In one study, Krabcova et al.[88] analyzed different methods of tissue sample preparation to vitrify hAM without using CPA and the results showed that the cooling of hAM in liquid ethane (−183°C) increases the percentage of epithelial cells viability significantly after re-warming. In another study, Moon et al.[89] developed a successful vitrification method using a combination of ethylene glycol and sucrose (40% v/v ethylene glycol, 18% v/v Ficoll 70, and 0.3 M sucrose) to maintain the morphological and biological properties in the amnion mesenchymal stem cells (such as expression of embryonic stem cell markers and potential of differentiation) after thawing.

4.1.3 | Freeze-drying

Lyophilization or freeze-drying is a method to dehydrate samples containing water as the main component. In this process, samples undergo a two-step process consisting of freezing and drying to sublimate frozen water (ice) by vacuum desiccation. The first freezing step could create irreversible damage to the tissues due to ice formation. After freezing, the samples are dried in two steps: first, the primary drying cycle is conducted at low pressure to generate sublimation of water and avoid the collapse of the tissue; and second, the secondary drying cycle is conducted including a slow increase of the temperature to gradually dehydrate the sample. In order to reduce damage, samples could be protected with a medium containing CPAs.[90,91] In this case, sugars as CPAs stabilize the proteins present in the tissue to avoid denaturation during the drying cycles.[92,93] Low probability of contamination, ease of storage at room temperature without the need for freezers or liquid nitrogen, and proper preservation of physical tissue properties are the advantages of this method.[94] The

section below (Section 4.2) provides additional information on freeze-dried hAM.

4.2 | Preservation and storage of native hAM

For the storage of hAM for clinical applications, the requirements of the underlying regulatory frameworks must be followed. In Europe, these are mainly recorded in the Directive 2004/23/EC[95] of the European Parliament and of the Council of March 31, 2004, that applies to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells intended for human use. Directive 2004/23/EC also lays down rules on the quality and safety of tissues and cells, for example, quality management, tissue and cell reception, processing, and storage conditions. The most crucial factor here is the safe application for the patient, which is based on the elimination of transmission of diseases, and on the elimination of contamination with the transplant. Furthermore, the efficacy of the tissue must be guaranteed even after storage. The processes applied during and after storage must be first assessed and approved by the competent authorities of each country. For the storage of clinically used hAM, all procedures described in Section 4.1. of this review are applied. In addition, hAM storage without CPAs and without controlled freezing at −80°C is also authorized for periods of time longer than 1 year (fresh frozen) as long as all quality criteria are met.[22] In routine clinical practice, various factors play a role in safe storage, such as the storage temperature and its duration.[96]

In this regard, the performance of hAM under different cryopreservation times has been researched by Wagner et al. performing studies at 0.5, 1, 3, and 6 months after cryopreservation with hAM stored at −80°C with glycerol, such as CPA, and glycerol-free. The results showed higher cell viability of samples cryopreserved in glycerol for up to 3 months of storage. No significant differences between glycerol containing and glycerol-free methods were observed at 6 months.[97] Nevertheless, if high cell viability is required, the application of CPAs is necessary to preserve cell viability, functionality as well as cell–cell and cell-matrix interactions. hAM cryopreserved with glycerol has been successfully employed as a scaffold for urothelium regeneration,[98] for melanocyte transplantation in patients with vitiligo[99] and wound healing in diabetic foot ulcer.[100] Cryopreserved hAM showed improved postoperative results in each treatment with a morphological structure similar to that of fresh one.[12] In another study, cryopreservation with DMSO preserved the hAM tissue with better in vitro functionality[101] and maintained the anti-angiogenic properties of the membrane.[102]

Alternatively, hAM preserved with the freeze-drying method has been successfully employed in the reconstruction of the ocular surface in rabbit models. The freeze-dried hAM did not show a lower performance of the biological properties such as anti-inflammation, antibacterial, and antifibroblastic activity compared to the membranes cryopreserved using 50% v/v glycerol solution at −80°C..[94,103,104] In other studies, hAM was pretreated with 10% v/v trehalose at 37°C for 2 hr[105] and raffinose (0.025 M, 1.0 M or 2.0 M) at 37°C for 2 hr[106] before freeze-drying, to evaluate the efficacy of sugars in terms of preservation and in terms of their safety for application of preserved membranes for ocular surface reconstruction. The results showed enhanced adaptability of the ocular surfaces for the pretreated and dried membranes in comparison with cryopreserved hAM due to better conservation of the basal membrane and collagen fibrils. Furthermore, the pretreatment step did not induce an inflammatory reaction and presented faster epithelization in rabbit sclera, as well as higher retention of factors such as TGF- *β1 and EGF*.

The performance of different biological properties of hAM was assessed after different preservation methods had been applied, as summarized in Table 2. The angiogenesis modulation in a rat model showed the same anti-angiogenic behavior between cryopreserved and fresh hAM for 6 months.[107] The anti-angiogenic property and the release of proapoptotic factors reduce the viability of cancer cells via apoptosis induction. This property was also preserved after cryopreservation using 50% v/v glycerol.[102] Other studies compared the effect of cryopreservation and freeze-drying process on hAM storage for 6 months compared to fresh hAM as a substrate for endothelial cells. Results showed that the preservation method has no significant effect on the mechanical behavior evaluated using a uniaxial tension test. Differences were observed in structural properties such as thickness and conservation of the basal membrane. Nevertheless, freeze-drying is suggested to be a desirable process to develop a substrate for endothelial cell culture. Niknejad et al.[6] showed that endothelial cell adhesion and cell viability could be significantly improved with an application of freeze-dried membranes.

The assessment of the antibacterial properties of hAM against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* after cryopreservation and freeze-drying showed that the presence of elafin as antibacterial peptide decreased in cryopreserved and freeze-dried samples. However, the antibacterial properties were maintained for all preservation methods. This indicates that antibacterial properties are not only related to elafin but also to other components of the ECM. The authors suggest that preserved hAM is a proper substitute for a fresh membrane.[108] In their work, a slow cooling protocol was used to study the cryoinjuries and cryoresistance of hAM. The authors compared tissues frozen with DMSO with a cooling rate of 1 K/min with fresh hAM and tissue frozen with liquid nitrogen without any CPA. Results indicate that metabolic activity (MTT assay) is reduced after cryopreservation using this approach, as compared to fresh hAM. Although being volume and equipment dependent, the authors noticed the temperature of ice formation to be −14°C and below.[109]

4.3 | Preservation of tissue-engineered products

The lack of availability of donated organs suitable for transplantation led to the development of TE to provide hospitals with functional ready-to-use TEPs to regenerate or replace damaged tissues or

TABLE 2 Properties evaluated after hAM preservation. (X = Not analyzed, ✓ = Analyzed)

	Preservation method			
Property assessed	Cryopreservation solution	Freeze-drying	Fresh hAM	Reference
Angiogenesis modulation	10% v/v DMSO 70% v/v PBS, 10% v/v FBS, 10% v/v DMEM, −80°C 6 months	×	✓	107
Anti-cancer activity	10% v/v DMSO 70% v/v PBS, 10% v/v FBS, 10% v/v DMEM, −80°C 6 months	×	✓	102
Substrate for endothelial cells	50% v/v glycerol 40% v/v DMEM, 10% v/v FBS −80°C 6 months	Prefrozen 30 min, dried −55°C for 24 hr	✓	6
Antibacterial effect	10% v/v DMSO 70% v/v PBS, 10% v/v FBS, 10% v/v DMEM, −80°C 6 months	Prefrozen 30 min, dried −55°C for 24 hr	✓	108
Cryoinjuries and cryoresistance	10% v/v DMSO DMEM, 10% v/v FBS Cooling 1 K/min to −80°C	×	✓	109

organs. In order to provide off-the-shelf availability of TEPs, their preservation must be considered. TEPs are formed by the growth of appropriate cells to regenerate bone, skeletal muscle, cartilage, and other tissues on biodegradable scaffolds made from synthetic and/or natural polymers.[110] In Section 3.2, different composites which can be created with hAM and different polymers are reviewed. Although several TE approaches have been developed and investigated to design appropriate hAM-based TECs, there are still no reports available in the literature on the elaboration of adequate storage processes for the preservation of hAM-based TECs with an indication of their clinical application. Therefore, the current available information on cryopreservation techniques applied for efficient storage of TECs composites employed in comparable fields of study (i.e., similar to hAM application) is reviewed below.

Usually, cells are frozen in suspension or as a monolayer.[111] Different studies have been conducted to demonstrate the cryopreservation of cells adhered to a substrate. In these studies, attention has been given to analyzing the effects of cryoprotectants composition and extracellular matrix design on cryopreservation outcome, for example, viability and functionality of the whole construct. The effect of directional freezing followed by gradual cooling on survival rates of adherent epithelial cells cultures was also analyzed in detail by Bahari et al..[112] In directional freezing, the container with cells or tissues in aqueous solution is moved down with a controlled velocity through a thermal gradient below the melting temperature, and the ice crystals grow in the opposite direction of movement in a directional way. The application of a thermal gradient allowed the preservation of a large volume of cells (around 500 ml), especially reproductive cells under a precise and uniform cooling rate. Moreover, avoiding the use of CPAs due to ice growing is controlled by the thermodynamic process described above.[113] In a preliminary test, epithelial cells from the cell line IEC-18 and Caco-2 were incubated with DMEM and different DMSO concentrations ranging from 0 to 10% v/v. The velocities tested in this test were 10, 30, and 90 μm/s equivalent to cooling rates 1.3, 3.8, and 11.3 K/min, respectively. After being directionally frozen with less than 2.5% v/v DMSO, IEC-18 cells presented injury after freezing independently on the applied cooling rate, while Caco-2 cells (10% v/v DMSO, 30 μm/s rate) presented normal cell shape and clear nuclei.

Few studies have been conducted on cryopreservation of complex structures having cell-matrix and cell–cell junction organizations. The primary challenge associated with cryopreservation of 3D constructs is that the cells adhered to or within substrates are more sensitive to cryoinjuries (osmotic stress, mechanical damage, nonhomogeneous heat, and mass transfer) and thus are more susceptible to damage caused by suboptimal cryopreservation conditions in comparison with suspended cells.[114] Due to the complex 3D geometry of the bulk tissue constructs, unequal heat and mass transfer makes it difficult to achieve appropriate optimal cooling and warming rates as well as homogeneous CPA distribution within a tissue.[74,115]

In a study conducted by Bissoyi et al.,[110] silk nanofibers obtained by electrospinning were seeded with MSCs from the umbilical cord to obtain TECs as promising constructs for bone and cartilage regeneration. The TECs were frozen using different cryoprotective agents such as natural osmolytes (trehalose and ectoin) in combination with DMSO using controlled cooling rates. The results showed that silk-based TECs cryopreserved in DMSO supplemented with trehalose and ectoin presented better cell viability than the cells frozen using sole DMSO with cell viability of 72 and 54%, respectively. Moreover, the cryopreserved scaffold showed unaltered mechanical properties and preservation of the cell cytoskeleton after freezing and thawing, concluding that the newly formulated relatively low toxic freezing

solution (2.5% v/v DMSO/0.04 M trehalose/0.04 M ectoin) is promising for successful preservation of silk-based TECs. In the work of Gurruchaga et al.,[116] the authors employed an allogeneic bioscaffold based on platelet-rich plasma and synovial fluid seeded with MSCs. The bioscaffold was cryopreserved in 10% v/v DMSO or the combination of 10% v/v DMSO and 0.2 M sucrose. The results show that the bioscaffolds preserved with a combination of DMSO and sucrose show a similar number of viable cells, and potential to differentiate into the chondrogenic lineage in comparison with fresh bioscaffolds that served as control.

Other comparisons between DMSO and sugars were made for the preservation of epithelial sheets for wound healing constructs.[117,118] An epidermal graft composed of chitosan and gelatin was seeded with human keratinocytes to create artificial skin. The graft was preserved with different CPA solutions containing 0.2 or 0.4 M trehalose and 10% v/v DMSO and frozen using a multi-step cooling profile (4°C for 30 min, −20°C for 2 hr and −80°C overnight followed by immersion in liquid nitrogen). The unfrozen and frozen grafts were transplanted to nude mice to analyze the efficacy of the cryopreserved construct for skin regeneration. After 14 days, post-transplantation trehalose-cryopreserved artificial skin repaired skin defects in a similar way to that of a non-cryopreserved control. Other groups showed that the application of trehalose-cryopreserved artificial skin resulted in enhanced wound closure.[117]

At the same time, application of pretreatment of TECs with sugars before freezing, such as sucrose, has been shown to enhance viability outcome after cryopreservation. On this subject, Mutsenko et al. validated an "in air" approach using porous 3D collagen-hydroxyapatite scaffolds seeded with amnion-derived MSCs[119] as a model of TECs. In this work, a positive effect of the incubation (pretreatment step) on cell-seeded scaffolds for 24 hr in 0.1 M sucrose was observed. Before freezing, the constructs were immersed in a CPA cocktail containing 10% v/v DMSO and 0.3 M sucrose, followed by the removal of excess of CPAs and slow freezing (1°C/min to −80°C, storage −152°C). This approach was defined as "in air" freezing. After 5 days of storage, samples were thawed following a two-step procedure: thawing at 37°C for 1 min and recultivation for 24 hr. Cell-seeded scaffolds treated with sucrose 24 hr before freezing presented a significantly higher viability of 80% in comparison to the samples frozen without sucrose (60% viability). Such method did not have a negative impact on scaffold composition and protein structure.

The freeze-drying technique was also employed to preserve tissue-engineered skin substitutes.[118] The tissue-engineered skin was incubated in 0.2 M trehalose and 10% v/v DMSO at 37°C for 8 hr. After the solution was removed, the constructs were frozen with a cooling speed of 1 K/min to −50°C, followed by drying under vacuum. Results showed that freeze-dried constructs presented a similar wound-healing effect in mice skin compared to standard cryopreservation. The authors suggest that freeze-drying is an advantageous method for the preservation and transport of this type of tissue-engineered skin at ambient temperature.

In summary, the TEPs preserved with sugars, such as sucrose and trehalose, presented better properties after storage than those preserved with DMSO alone. Moreover, an additional pretreatment step including an application of sugars should be considered for improved viability and functionality for the materials to be preserved. Table 3 summarizes cryopreservation of TEPs, and further research can be consulted in the review for personalized TE therapies.[120]

5 | APPLICATIONS

Native hAM has been applied as a scaffold for TE and regeneration in different medical fields (the reader can find this information in previous sections). Some reviews describe its use as a grafting material,[8] in oral and periodontal surgeries,[23] cartilage damage,[7] lower extremity repair,[121] healing of chronic wounds and ulcers,[122] as a biomaterial in urology,[123,124] in gynecology,[125] as of patch for cardiac surgery,[126] and as a treatment of ocular surface pathologies.[29]

In ophthalmology, the transplantation of frozen or fresh hAM is often used for pterygium excision, conjunctival surface reconstruction (e.g., after symblepharon lysis), reepithelialization in corneal ulcer, or for temporary closure of perforated corneas. Most frequently, it is used for improved healing of corneal ulcers.[127] Transplantation techniques can be classified into three types: graft, patch, or sandwich. In the grafting technique, the hAM is placed with the epithelium side facing up the defect tissue, for example, the cornea.[128] Before transplantation, the surrounding 1–2 mm of the host corneal epithelium is debrided. Regenerating epithelium grows over the membrane, whereas hAM (Figure 5(a)) is mostly incorporated into the host tissue. In deep-rooted corneal ulcer multilayer graft inlays can be sutured. In the patch (or overlay) technique, the membrane is sutured to the surrounding conjunctiva. The membrane is larger than the underlying defect, so the host epithelium is present below the membrane. The epithelium side is commonly placed facing the defect tissue. Hence, the fragile epithelium can grow under membrane protection. The sandwich technique combines the graft, and the patch technique, as shown in Figure 5(b). Inner layers are attached as a graft, and for protection, an outer layer is fixed as a patch. The epithelium is expected to grow under the patch but over the uppermost inlay graft. The layers are attached to the cornea by non-absorbable sutures, or to other tissue with absorbable and non-absorbable sutures. A contact lens is additionally often inserted for the protection of the thin amniotic membrane.[129] Also, to avoid complications with the sutures, a sutureless fixation alternative *AmnioClip-plus* (Figure 5(c)) is employed. Moreover, the hAM is mounted between two rings and applied like a large contact lens on the ocular surface.[55]

In addition, the efficacy of commercially available hAM has been tested in ocular treatments for patients. According to Finger et al.,[53] the product *Amnioguard* (Bio-Tissue, USA) is a super-thick amnion ideal for ocular surface reconstruction (ST-AMGs). Eleven patients were treated with ST-AMGs implants after resection of ocular tumors with cryotherapy. Most patients (75%) showed partial graft dissolution during the third week after transplantation and complete epithelization without a wound. After 25 months, 83% of the patients

TABLE 3 Cryopreservation of TECs

Material preserved	Cryoprotectants used	Cryopreservation method	Reference
MSCs from umbilical cord seeded on electrospun nanofibrous silk fibroin scaffolds forming a TECs	1. 10% v/v DMSO with 50% v/v FBS 2. 0.04 M trehalose 3. 0.04 M ectoin 4. 0.04 M trehalose and 0.04 M ectoin 5. 0.04 M trehalose, 0.04 M ectoin 100 lg catalase 6. 0.04 M trehalose, 0.04 M ectoin and 2.5% (v/v) DMSO 7. 0.04 M trehalose, 0.04 M ectoin, 2.5% v/v DMSO and 100 lg catalase as antioxidant All solutions were prepared with DMEM media	Exposition with CPAs for 10 min at 4°C. freezing at −1 K/min to −150°C and storage in liquid nitrogen	110
Allogeneic biomimetic scaffold seed with MSCs	1. 10% v/v DMSO 2. 10% v/v human serum 3. 10% v/v DMSO +10% v/v human serum 4. 0.2 M sucrose, 10% v/v DMSO +0.2 M sucrose 5. 10% v/v human serum +0.2 M sucrose 6. 10% v/v DMSO +10% v/v human serum +0.2 M sucrose CPA solutions were prepared by the combination of DMSO, sucrose, and human serum	20 min on ice, overnight at −80°C on a container and last store into liquid N_2 tanks for at least 3 weeks before performing any assay.	116
Epithelial sheets	Four cryopreservation solutions 1. 0.2 M trehalose, 10% v/v DMSO, 50% v/v fetal bovine serum 2. 0.4 M trehalose, 10% v/v DMSO, 50% v/v FBS 3. 0.6 M trehalose, 10% v/v DMSO, 50% v/v FBS 4. 10% v/v DMSO, 50% v/v FBS (group DMSO, as a negative control)	Samples immersed in cryopreservation solutions for 30 min 4°C, after placed in liquid nitrogen. Control samples step frozen 4°C for 30 min, after −20°C for 2 hr,−80°C overnight and then placed into liquid nitrogen for 1 month	117
Amnion derived MSCs seeded on 3D collagen scaffolds	1. 10% v/v DMSO, 20% v/v FBS 2. 10% v/v DMSO, 20% v/v FBS, 0.3 M sucrose	Cell- seeded scaffolds pretreated with 0.1 M sucrose for 24 hr after immersed in cryoprotective solution in ice for 15 min. Removal of CPAs and freezing at 1°C/min to −80°C. stored −152°C for 5 days before thawing	119
Fibroblasts freeze-dried to be seeded in collagen for TECs	1. 0.2 M trehalose +10% v/v DMSO 2. 0.2 M trehalose 3. 10% v/v DMSO Fresh cell culture medium acted as a control	Freezing with 1 K/min to −50°C freeze-dried for 7 hr at −50°C. Stored in vacuum at room temperature 1 week before use	118

showed complete local tumor control. The product *ProKera*® from the same company was evaluated by Morkin et al.[54] for the treatment of neuropathic corneal pain (NCP) in nine patients. The results showed that pain severity decreased in around 70% of the patients. The paintable sensation decreased from 7 to 2 points on a scale from 1 to 10 after 2 days of treatment. The results suggest that *Prokera*®

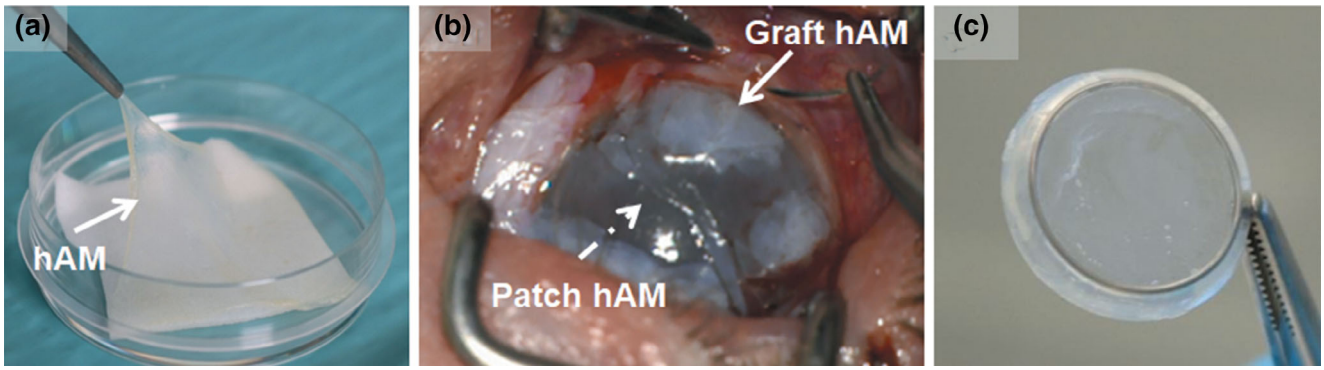

FIGURE 5 Amniotic membrane transplantation in a severely burned eye after an explosion, (a) hAM fragment on a carrier, (b) Sandwich transplantation technique, (c) Alternative sutureless product AmnioClip-plus

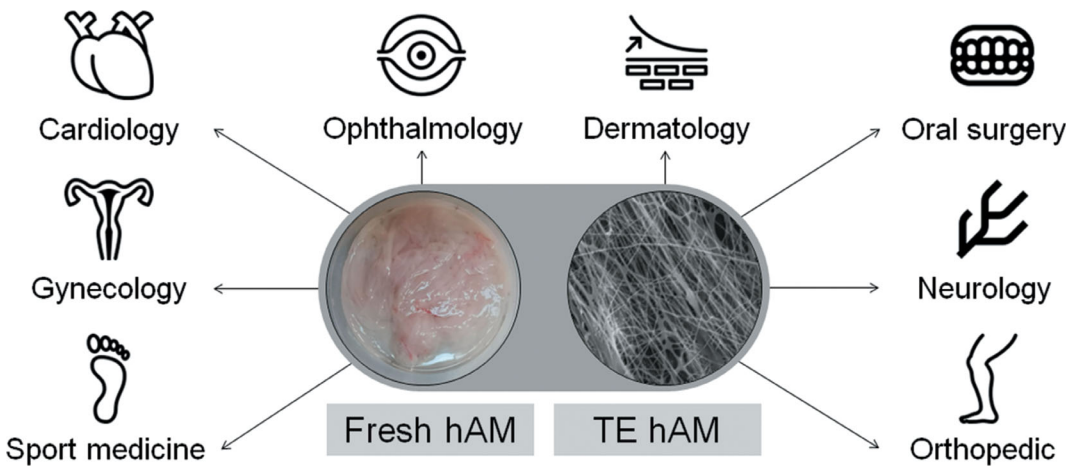

FIGURE 6 Application fields for fresh and tissue-engineered (TE) hAM

provides safe and effective treatment to achieve pain control in patients with NCP.

Along with its effectiveness in ophthalmology, hAM grafts have been employed in the cardiovascular field. Amensag et al.[57] constructed a small-diameter (3.2 mm diameter) vascular graft from rolled human amniotic membrane and evaluated mechanical properties and viability in vivo in a rabbit model in the early post-implantation period. After 4 weeks, the constructed graft showed evidence of active cellular remodeling and did not present hyper-acute rejection or thrombotic occlusion. The vascular graft provided a supportive scaffold for the cardiac cell population in vivo. By this approach, it would be possible to create patient-specific vascular grafts having the desired dimensions and to lower the rate of rejection.

On the other hand, hAM-based TE scaffolds have shown improved performance in vitro and in vivo. Portmann-Lanz et al.[130] proposed a cell-free extracellular matrix scaffold obtained from hAM to design material for amnion cell outgrowth used to repair prematurely ruptured fetal membranes. The hAM scaffold was decellularized by enzymatic treatment and manual surface scraping. Afterwards, the scaffold was recellularized with amnion mesenchymal and epithelial cells to assemble a TEC. The scaffold presented a porous collagen fiber network with the appropriate biomechanical properties for cell growth. The efficacy of the hAM scaffold was tested in comparison with a polyester-urethane scaffold to analyze the wound healing response in sealing fetal membrane defects in a rabbit model. As a result, the hAM products sealed the fetal membrane defects better than the artificial scaffold, showing abundant reepithelization and lower local inflammation.[56]

Hortensius et al.[58] proposed collagen-glycosaminoglycan scaffolds supplemented with hyaluronic acid or amniotic membrane fabricated by freeze-drying technique. The aim was to analyze the scaffold immunomodulatory effects during tendon regeneration. The in vitro results with tenocytes after 7 days of culture showed increased metabolic activity for the scaffolds with hAM in a pro-inflammatory environment with interleukin-1 beta. According to the authors, the inclusion of hyaluronic acid or hAM in the scaffolds could modify the inflammatory response associated with scar formation due to less TGF-β1 release. In other research, Gholipourmalekabadi et al.[59] developed an artificial skin 3D bi-layer scaffold made of biological decellularized hAM with viscoelastic electrospun nanofibrous silk fibroin (ESF) spun on

top. The hAM/ESF scaffold and hAM alone were tested with adipose tissue-derived mesenchymal stem cells. The results showed increased expression of a vascular endothelial growth factor and primary fibroblast growth factor for the cultured hAM/ESF in comparison with hAM alone, suggesting that the scaffold could potentially be applied in a clinical setting for skin regeneration. In summary, Table 1 highlights the state-of-the-art information on the application of hAM-based composites.

6 | SUMMARY

The research of the human amniotic membrane has been a trending topic for decades. The unique characteristics of hAM are responsible for its wide range of applications, as summarized in Figure 6. There are several commercial products available with applications in wound healing and corneal treatment. One of the critical steps for the preservation of its properties is adequate handling and storage. Different alternatives analyzed for preservation present similar results between the native hAM, freeze-dried, and cryopreserved hAM. The right storage technique will depend on the available facilities and the desired application.

The amniotic membrane is an ideal scaffolding material based on its composition and the ease of collection from the human placenta. However, the differences between donors should be carefully considered to guarantee the standardization of different properties. The preparation of an extract or powder from native hAM and its combination with biodegradable and FDA approved polymers is a promising method to develop tissue-engineered hAM-based constructs presenting suitable properties for different applications. Nonetheless, the adequate preservation technique to ensure the availability of these TEPs for clinical applications should still be explored in detail.

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CONFLICT OF INTEREST

The authors Kern T (until 2019) and Framme C are part of the medical staff of Clinic for Ophthalmology, Hannover Medical School, and practice corneal treatment with human amniotic membrane. Hofmann N and Börgel M are staff members of the German Society for Tissue Transplantation with non-financial interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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